Soil carbon dioxide and methane fluxes as affected by tillage and N fertilization in dryland conditions

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Abstract

Background and Aims. The effects of tillage and N fertilization on CO_2 and CH_4 emissions are a cause for concern worldwide. This paper quantifies these effects in a Mediterranean dryland area.

Methods. CO_2 and CH_4 fluxes were measured in two field experiments. A long-term experiment compared two types of tillage (NT, no-tillage, and CT, conventional intensive tillage) and three N fertilization rates (0, 60 and 120 kg N ha^{-1}). A short-term experiment compared NT and CT, three N fertilization doses (0, 75 and 150 kg N ha^{-1}) and two types of fertilizer (mineral N and organic N with pig slurry). Aboveground and root biomass C inputs, soil organic carbon stocks and grain yield were also quantified.

Results. The NT treatment showed a greater mean CO_2 flux than the CT treatment in both experiments. In the long-term experiment CH_4 oxidation was greater under NT, whereas in the short-term experiment it was greater under CT. The fertilization treatments also affected CO_2 emissions in the short-term experiment, with the greatest fluxes when 75 and 150 kg organic N ha^{-1} was applied. Overall, the amount of CO_2 emitted ranged between 0.47 and 6.0 kg CO_2-equivalent kg $grain^{-1}$. NT lowered yield-scaled emissions in both experiments, but these treatment effects were largely driven by an increase in grain yield.

Conclusions. In dryland Mediterranean agroecosystems the combination of NT and medium rates of either mineral or organic N fertilization can be an appropriate strategy for optimizing CO_2 and CH_4 emissions and grain yield.

Keywords

Carbon dioxide, Mediterranean dryland, methane, nitrogen fertilization, tillage, soil organic carbon, yield-scaled GHG emissions.

Introduction

The agricultural sector is responsible for 10%-12% of the global anthropogenic emissions of greenhouse gases (GHG) (Smith et al. 2007). This effect could be greatly mitigated through a proper choice of crop and land management systems. Moreover, optimized agronomic practices can also increase the soil C sink, a climate change mitigation measure that was proposed in the Kyoto Protocol (United Nations, 1998) (Smith, 2004). There is therefore a great need to identify agricultural practices with the smallest GHG emission footprints while maintaining high crop productivity.

Carbon dioxide (CO_2) and methane (CH_4) are two of the most important GHG owing to their global warming potential (GWP) and long residence time in the atmosphere (IPCC, 1995). Soil emission of CO_2 from agricultural systems causes a loss of soil organic carbon (SOC) that directly affects the fertility and sustainability of soils (Davidson and Janssens, 2006). Soil CO_2 emissions are the result of SOC mineralization and root respiration processes. However, only the mineralization process represents a net C loss from the soil to the atmosphere owing to its heterotrophic nature (Morell et al. 2012). Agricultural soils can also act as net emitters or oxidizers of CH_4, depending on the relative balance of the methanotrophic and methanogenic processes (Hütsch, 2001). The methanotrophic process involves the microbial oxidation of CH_4 in aerobic conditions while the methanogenic process entails the anaerobic digestion of soil organic matter (Le Mer and Roger, 2001). Although those processes can occur simultaneously in arable ecosystems, upland soils usually act as net CH_4 oxidizers (Conrad, 1995).

Tillage and N fertilization of cropland imply a significant investment by farmers and therefore have great potential for optimization. They also play a major role in the mechanisms that drive the production, transport and consumption of GHG in soils. During tillage operations, soil structure is greatly disturbed and the CO_2 contained in the soil pore system is lost due to a process known as degassing (Reicosky et al. 1997). In addition to this physical release of gas, tillage also affects soil microbial activity through changes in substrate availability and micro-environmental conditions. For instance, tillage buries crop residues, thus increasing the contact between C-rich substrates and soil particles where greater soil moisture and nutrients are available (Balesdent et al. 2000; Paustian et al. 1997). Tillage also accelerates the breakdown of soil aggregates, thus releasing the organic carbon protected within them and increasing its availability to soil microorganisms (Beare et al. 1994). Tillage can also influence the processes that regulate the emission and/or consumption of CH_4 through its impact on the soil water regime, soil structure and microbial diversity. For instance, Ball et al. (1999) hypothesized that no-tillage (NT) increases the oxidation of CH_4 because of the absence of soil disturbance, greater gas diffusivity, and the reduction of damage to CH_4 oxidizers compared with conventional tillage (CT). This hypothesis has been tested in field experiments, in which a greater (Ball et al. 1999; Kessavalou et al. 1998) or equal (Alluvione et al. 2009; Piva et al. 2012; Sainju et al. 2012) CH_4 oxidative capacity was found under NT than under CT. Venterea et al. (2005) reported an interaction between tillage and N fertilization treatments, with greater or lower CH_4 oxidation under NT depending on the type of fertilizer.

The application of nitrogen fertilizer affects soil C stocks and GHG emissions. In a semiarid area of NE Spain, Morell et al. (2011) found an increase in the amount of C sequestered in the soil after 15 years of mineral N fertilizer application as a result of greater crop residue production. The same authors also found greater CO_2 emissions when mineral N was applied in wet years, but no differences between fertilized and unfertilized treatments in dry years. The type of fertilizer applied also has a great influence on soil CO_2 emissions (Ding et al. 2007). In turn, several studies (e.g. Hütsch et al. 1993) have shown that the addition of N reduces the uptake of atmospheric CH_4 by soil. This finding has been mainly related to a direct inhibition of CH_4 oxidation by ammonium in the soil (Conrad, 1996; Whittenbury et al. 1970). When slurries (e.g. pig slurry) are used as fertilizers, soil respiration is enhanced and can promote anaerobic microsites, thus reducing the oxidation of CH_4 and increasing its production (Meijide et al. 2010).

The main characteristic of rainfed Mediterranean agroecosystems is the lack of water available for crop growth (Cantero-Martínez et al. 2007), a limiting factor that also affects crop response to N fertilization (Cantero-Martínez et al. 2003; Ryan et al. 2009). Although the benefits of irrigation in terms of crop productivity in Mediterranean areas have been clear since ancient history, the scarcity of water usually prevents the establishment of new irrigated areas.

In Mediterranean Spain, the use of reduced tillage or no-tillage techniques has been suggested as a promising strategy for increasing the amount of SOC because higher soil water conservation and greater physical protection of carbon within soil aggregates under NT in comparison with CT lead to an increase in C inputs (Cantero-Martínez et al. 2007; Álvaro-Fuentes et al. 2008a). Furthermore, the application of animal waste to agricultural soils is a common practice in Mediterranean Spain because of the intensive animal production in the area (Yagüe and Quílez, 2013).

Prior to the present study, Morell et al. (2011) studied the effect of different types of tillage and rates of mineral N on CO_2 emissions in the same semiarid area. Also, Meijide et al. (2010) quantified the emissions of both CO_2 and CH_4 under different types of organic and mineral fertilization. However, in the literature there is a lack of CH_4 data to better understand C cycling in agroecosystems. To date, no studies have been conducted in the Mediterranean area to investigate the impact of different tillage, N rates and fertilizer types on the fluxes of CH_4 and CO_2, including their effect on the yield-scaled emissions of those gases, in order to evaluate their efficiency in terms of GHG emitted per unit of grain mass produced.

Therefore, our objective was to quantify the interactive effects of tillage and N fertilization type and rate on the emission of CH_4 and CO_2 as well as on biomass C inputs and SOC stocks, in order to identify environmentally sustainable practices while maintaining crop yields. We hypothesized that the interaction between tillage and N fertilization practices would affect crop performance and soil microbial activity and, consequently, GHG emission and soil C storage in Mediterranean rainfed cropping systems.

Material and Methods

Sites and treatments

Long-term experiment

A tillage and mineral N fertilization experiment was established in 1996 in Agramunt, NE Spain (41º48’36’’N, 1º07’06’’E, 330 masl). The climate in the area is Mediterranean temperate, with mean values of annual precipitation, annual air temperature and annual reference evapotranspiration (FAO Penman-Monteith methodology) of 430 mm, 13.8 ºC and 855 mm, respectively. The soil was classified as Typic Xerofluvent (Soil Survey Staff, 1975). Selected soil properties at the start of the experiment in the 0-30 cm layer were as follows: pH (H_2O, 1:2.5) was 8.5; electrical conductivity (1:5) was 0.15 dS m^{-1}; organic C concentration was 7.6 g kg^{-1}; and sand (2000-50 μm), silt (50-2 μm) and clay (<2 μm) content were 465, 417 and 118 g kg^{-1}, respectively. Two types of tillage (no-tillage and conventional intensive tillage with a moldboard plow) and three mineral N fertilization rates (0, 60 and 120 kg N ha^{-1}) were compared in a randomized block design with three replications. The medium N rate (60 kg N ha^{-1}) was chosen according to the productive potential in the area, while the high N rate (120 kg N ha^{-1}) was chosen because it was the most common among farmers. Plot size was 50 x 6 m. The NT treatment consisted of a total herbicide application (1.5 L 36% glyphosate per hectare) to control weeds before sowing. The CT treatment consisted of one pass of a moldboard plow to 25 cm depth followed by one or two passes of a cultivator to 15 cm depth, both performed in September-October. Mineral N fertilizer was applied manually and split into two applications: one-third of the dose as ammonium sulphate (21% N) before seeding and the rest of the dose as ammonium nitrate (33.5% N) at the beginning of tillering in February. The cropping system consisted of a barley monocropping (with *Hordeum vulgare* L.*;* cv. Hispanic in the 1996-2010 period and cv. Cierzo in the 2010-2013 periods), which is a traditional system in the area. Planting was performed in November with a direct drilling machine with disk openers set to 2-4 cm depth and 17 cm between rows. Harvesting was carried out with a commercial medium-sized combine in June. The straw residue was chopped and spread over the soil by the same machine. The historical management of the field prior to the establishment of the experiment was based on conventional intensive tillage with moldboard plowing and winter grain cereal monoculture.

Short-term experiment

An experimental field was established in Senés de Alcubierre, NE Spain (41º54'12''N, 0º30'15''W, 395 masl) in 2010. Mean values of annual precipitation, annual air temperature and annual evapotranspiration (FAO Penman-Monteith methodology) in the area are 327 mm, 13.4 ºC and 1197 mm, respectively. The soil was classified as Typic Calcixerept (Soil Survey Staff, 1975). Selected soil properties at the start of the experiment in the 0-30 cm depth were as follows: pH (H_2O, 1:2.5) was 8.0; electrical conductivity (1:5) was 1.04 dS m^{-1}; organic C (g kg^{-1}) was 15.6; organic N (g kg^{-1}) was 1.4; and sand (2000-50 μm), silt (50-2 μm) and clay (<2 μm) content were 62, 633 and 305 g kg^{-1}, respectively. The cropping system before and during the experiment consisted of a barley (cv. Meseta) monoculture. During the four years prior to the set-up of the experiment, soil management consisted of NT with mineral N fertilizer additions at rates of 75-100 kg ha^{-1}. Before that period two passes with a subsoiler or a chisel had been used since the 1970s.

Two tillage systems (CT, with two passes of chisel plowing, and NT), three N fertilization doses (0, 75 and 150 kg N ha^{-1}) and two types of fertilizer products (mineral N and organic N with pig slurry) were compared. The highest N rate (150 kg N ha^{-1}) was chosen according to the most common dose applied by farmers in the area, while the medium rate (75 kg N ha^{-1}) was chosen to evaluate the possibility of reducing the N application rate without compromising crop yields. The NT treatment consisted of a total herbicide application (1.5 L 36% glyphosate per hectare) to control weeds before sowing. Mineral N fertilizer was applied manually. The treatment with 150 kg N ha^{-1} was split into two applications: half of the dose as ammonium sulphate (21% N) before tillage and the other half as ammonium nitrate (33.5% N) at the beginning of tillering in February. For the 75 kg N ha^{-1} treatment the entire dose was applied as ammonium nitrate at tillering. Likewise, in the treatments with organic fertilization, the 75 kg N ha^{-1} rate was applied entirely at tillering and the 150 kg N ha^{-1} rate was split into two applications of 75 kg N ha^{-1} each, one before tillage and the other one at tillering. The organic fertilization treatment consisted of the application of pig slurry from a commercial farm of the area. The slurry was conventionally surface-spread using a commercial vacuum tanker fitted with a splashplate. The machinery was previously calibrated to apply the precise dose after analyzing the pig slurry composition. The main characteristics of the pig slurry applied during the whole experimental period are shown in Table 1. As in the long-term experiment, planting was performed in November with a direct drilling machine with disk openers set to 2-4 cm depth and 17 cm between rows and harvesting was carried out with a commercial medium-sized combine in June. The straw residue was chopped and spread over the soil by the same machine. The experiment consisted of a randomized complete block design with three replications. Plot size was 40 x 12 m in the organic N fertilization treatment and 40 x 6 m in the mineral N fertilization treatment.

For both experiments, air temperature and rainfall observations were recorded on a daily basis using an automatic weather station located at each experimental site.

Gas sampling and analyses

CO_2 and CH_4 emissions were measured every two or three weeks with the non-steady-state chamber methodology (Hutchinson and Mosier, 1981). Additional gas measurements were made the day prior to fertilizer application and 4 and 72 hours after the application. The measurement period covered three cropping seasons (2010-2011, 2011-2012 and 2012-2013) in the short-term experiment and two cropping seasons (2010-2011 and 2011-2012) in the long-term experiment. Samplings were also performed during the summer-autumn fallow period (June-November) in order to quantify emissions for the entire year. However, owing to methodological constraints, the first gas samplings started in both experiments in February 2011 at the time of top-dressing fertilizer application.

At the beginning of both experiments, two polyvinyl chloride rings (31.5 cm internal diameter) per plot were inserted 5 cm into the soil of each experimental plot. The rings were only removed at the time of tillage, planting and harvesting operations, allowing a minimum lapse of 24 hours following ring rearrangement at the initial location before any gas sampling to avoid the concomitant effects of soil disturbance on gas emissions. Polyvinyl chloride chambers (20 cm height) were fitted into the rings when measurements were performed. A polytetrafluoroethylene vent (10 cm long and 0.4 cm internal diameter) was installed on one side of the chambers to prevent possible changes in pressure during the deployment of chambers and gas sampling. The chambers were covered with a reflective insulation fabric (model Aislatermic, Arelux, Zaragoza, Spain) that consisted of two reflective layers of aluminum film bonded to an inner layer of polyethylene bubbles in order to diminish internal increases in temperature. A metal fitting was attached in the center of the top of the chamber and lined with two silicon-Teflon septa as a sampling port.

Soil gas samples (15 mL) were obtained with polypropylene syringes at 0, 30 and 60 minutes after closing the camber and injected into 12-mL Exetainer® borosilicate glass vials (model 038W, Labco, High Wycombe, UK). Each block of the experiment (i.e., 10 treatments) was sampled by one operator in order to reduce as much as possible the amount of time during the sampling process, thus avoiding temperature-induced biases (Rochette et al. 2012). Gas samples were analyzed with an Agilent 7890A gas chromatography system equipped with a flame ionization detector + methanizer and two valves in order to obtain the gases of interest (i.e., CH_4 and CO_2) for each gas injection. A HP-Plot Q column (30 m long, 0.32 mm in section and 20 μm thick) was used, with a 15-m-long pre-column of the same characteristics. The injector and the oven temperatures were set to 50 ºC. The temperatures of the flame ionization detector and the methanizer were set to 250 and 375 ºC, respectively. For the detector, H_2 was used as a carrier gas and N_2 as a make-up gas at 35 and 25 mL min^{-1}, respectively. The volume of sample injected was 1 mL. The system was calibrated using ultra-high purity CH_4 and CO_2 standards (Carburos Metálicos, Barcelona, Spain). Emission rates were calculated taking into account the linear increase in the gas concentration within the chamber over the sampling time and correcting for the air temperature.

Biomass sampling and analyses

In the short-term experiment, crop aboveground biomass was measured right before the harvest in the three growing seasons studied by cutting the plants at the soil surface level along 0.5 m of the seeding line at three randomly selected locations per plot. Taking into account that the distance between seeding lines was 0.2 m, the sampled area in each plot was 0.3 m^2. The samples were dried at 65 ºC for 48 h and weighed. Then the dried samples were threshed and the grain was weighed. Aboveground biomass per unit of area was calculated by dividing the weight of the aboveground biomass excluding the grain by the area sampled.

Root biomass was measured at flowering in April 2012 and in May 2013 in the short-term experiment. For each plot, four soil cores (0-30 cm) were obtained, two in the seeding line and two between lines. Special care was taken to avoid wheel track locations. Each soil sample was dispersed with a 5% sodium hexametaphosphate solution in a reciprocal shaker for at least 30 minutes and then washed by hand with a low-pressure shower jet through a 0.5-mm sieve to recover the roots, following the methodology proposed by Böhm (1979). Once washed, the sieve was submerged in a tray filled with water in order to ease the skimming of the roots. Finally, the roots were oven-dried at 65 ºC and weighed. Root biomass per unit of area was calculated by dividing the weight of roots by the area sampled with the core. Afterwards, above- and belowground biomass samples were analyzed for C content by dry combustion. The above- and belowground biomass C inputs were calculated by multiplying the weight of each fraction of biomass by its C content.

Grain yield of each treatment was measured in 2012 in the long-term experiment and in 2011, 2012 and 2013 in the short-term experiment by harvesting the plots with a commercial combine and weighing the grain. After determining the grain moisture content, grain yield was corrected to 10% moisture.

Soil sampling and analyses

Soil samples from the 0-5 cm soil layer were collected on each sampling date near each gas sampling chamber. Water-filled pore space (WFPS) was calculated as the quotient between soil volumetric water content and total porosity. The volumetric water content was calculated as the gravimetric water content times the soil bulk density. The gravimetric water content was obtained by oven-drying the soil samples at 105 ºC for the long-term experiment and at 50 ºC for the short-term experiment until constant weight. In the short-term experiment, soil was dried at 50 ºC in order to avoid the dehydration of the gypsum present in the soil in this experiment (Porta, 1998). Soil porosity was calculated as a function of soil bulk density assuming a particle density of 2.65 Mg m^{-3}. Soil bulk density was determined using the cylinder method (Grossman and Reinsch, 2002). Moreover, on each gas sampling date, soil temperature was measured at 5 cm soil depth with a hand-held probe.

In the short-term experiment, a soil sampling was performed at the end of the experiment (June 2013) to quantify SOC stocks. Two sampling areas per plot were selected and soil samples were taken from the whole soil profile at five depths: 0-5, 5-10, 10-25, 25-50 and 50-75 cm. For the same depths, soil bulk density was determined using the cylinder method (Grossman and Reinsch, 2002). Once in the laboratory, the samples were 2-mm sieved and then air-dried. The SOC concentration was determined using the dichromate wet oxidation method of Walkley and Black described by Nelson and Sommers (1996). During the oxidation, extensive heating at 150 ºC for 30 minutes was used in order to increase the digestion of SOC (Mebius, 1960). Finally, the SOC stock was calculated using the equivalent soil mass procedure proposed by Ellert and Bettany (1995).

Calculations and data analysis

For both experiments, the cumulative soil C loss and gain due to the fluxes of CO_2 and CH_4, respectively, during the whole experimental period were quantified on a mass basis (i.e., kg C ha^{-1}) using the trapezoid rule. Also, for both experiments the yield-scaled net fluxes of CH_4 and CO_2 were calculated and expressed in terms of kg of CO_2 equivalent emitted per kg of grain produced. In the long-term experiment, this ratio was calculated for the 2011-2012 growing season by integrating the emissions of CH_4 and CO_2 from the pre-seeding application of fertilizers until the harvest of the crop, taking into account that CH_4 has a GWP 25 times greater than CO_2 (Forster et al. 2007), and dividing that result by the amount of grain produced by each treatment in that season. The ratio was also calculated in the short-term experiment for the 2011-2012 and 2012-2013 growing seasons by integrating the emissions of CH_4 and CO_2 from the pre-seeding application of fertilizers in the 2011-2012 growing season until the harvest in the 2012-2013 season and dividing that result by the sum of grain produced by each treatment in both cropping seasons.

For each site, data for WFPS and CO_2 and CH_4 fluxes were analyzed using the SAS statistical software (SAS institute, 1990) to perform a repeated measures analysis of variance (ANOVA). ANOVAs for the cumulative C losses of the two gases, the aboveground and root biomass C inputs, the SOC stocks, and the yield-scaled ratios between the C-gases emitted and the grain produced were also performed. When significant, differences between treatments were identified at 0.05 probability level of significance using a Tukey test. A stepwise regression was performed with the JMP 10 statistical package (SAS Institute Inc., 2012) to test the presence of relationships between CH_4 and CO_2 fluxes and soil WFPS and temperature at 0-5 cm soil depth.

Results

Environmental conditions and soil WFPS during the experiments

Rainfall and air temperature for the 2010-2011, 2011-2012 and 2012-2013 cropping seasons are shown in Figure 1. At both sites a large variation in precipitation was recorded during the three cropping seasons, as expected in our Mediterranean conditions. Annual rainfall ranged from 211 to 530 mm and from 280 to 537 mm in the long-term and short-term experiments, respectively. In the long-term experiment (Fig. 1 A), precipitation was lower than the 30-year average for the area (430 mm) in the 2010-2011 and 2011-2012 cropping seasons but higher in the 2012-13 season. In the short-term experiment (Fig. 1 B), precipitation was lower than the 30-year average (327 mm) in the 2011-12 cropping season, but exceptionally high in the 2012-13 season, particularly in the autumn and spring months. Air temperature showed the highest values during the summer months (June-August) and the lowest during the winter months (December-February). Over the experimental period, soil temperature ranged from -1.3 to 29.1 ºC in the long-term experiment (Fig. 2A) and from 1.4 to 29.3 ºC in the short-term one (Fig. 2 B). For both experiments, soil temperature was below 15 ºC during the applications of fertilizers except in the pre-seeding application of the 2011-2012 cropping season in the short-term experiment, when soil temperature reached 23.7 ºC (Fig. 2 B).

In both experiments, tillage significantly affected WFPS (Fig. 3 and Tables 2 and 3). In the long-term experiment, mean WFPS values were 19.8% and 44.1% for the CT and NT treatments, respectively (Table 2), while in the short-term experiment mean WFPS for the same treatments was 18.5% and 32.0%, respectively (Table 3). For both experiments NT had greater WFPS than CT on most sampling dates (Fig. 3). On the other hand, neither the nitrogen treatments nor the interaction between tillage and nitrogen significantly affected WFPS (Table 2).

Tillage and N fertilization effects on CH_4 emissions

In the long-term experiment, greater net uptake of CH_4 was observed under NT (2.4 kg CH_4-C ha^{-1}) than under CT (1.1 kg CH_4-C ha^{-1}) (Table 2), with no interaction between time and tillage treatment (data not shown). By contrast, in the short-term experiment greater mean CH_4 oxidation was found under CT (2.7 kg CH_4-C ha^{-1}) than under NT

(1.2 kg CH_4-C ha^{-1}) (Table 3). Moreover, in this experiment, the temporal dynamics of the CH_4 fluxes was affected by tillage, with higher emission peaks of CH_4 under NT than under CT for two of the five fertilizer applications (Fig. 4A). Also, for both CT and NT, a net emission of CH_4 from the soil to the atmosphere occurred during the coldest months (December-February) (Fig. 4A). In the long-term experiment, net emissions of CH_4 were observed in six and four sampling dates for CT and NT, respectively (data not shown).

No significant effects of mineral N rate on the dynamics of CH_4 fluxes were found in the long-term experiment. However, net uptake of CH_4 tended to decrease with increasing fertilizer N rates (Table 2). In the short-term experiment, although no differences between fertilization treatments were noted in the mean values of CH_4 fluxes, N fertilization affected the dynamics of CH_4 fluxes, with significant differences on six dates, four of them coincident with fertilizer applications (Table 3, Fig. 5A). Moreover, a significant (r^2: 0.27; P<0.001) logarithmic relationship was found between CH_4 fluxes and soil temperature in the long-term experiment (Fig. 6). On the other hand, no significant relationship was found between CH_4 fluxes and soil gravimetric moisture content (data not shown). In the short-term experiment no correlations were found between soil variables and GHG emissions.

Tillage and N fertilization effects on CO_2 emissions

Tillage significantly affected the average CO_2 emissions in the entire period studied, with a greater mean CO_2 flux under NT than under CT in both experiments (Tables 2 and 3). In the long-term experiment, soil CO_2 fluxes ranged between 91.50 and 1872.18 mg CO_2-C m^{-2} d^{-1} and were higher in the summer months (June-September) than in the winter months (December-March) (Fig. 7). Greater CO_2 fluxes were observed under NT than under CT on most sampling dates (Fig. 7). In the short-term experiment, a trend of higher CO_2 emissions during the fast-growing period of the crop (February-May) was observed for both tillage treatments (Fig. 4B). As in the long-term experiment, significant differences between tillage treatments were found for most of the sampling dates, with greater values under NT than under CT (Fig. 4B).

In the long-term experiment, the mineral N rates applied did not affect soil CO_2 emissions (Table 2). On the other hand, fertilization treatments affected CO_2 emissions in the short-term experiment (Fig. 5B). In this case, the application of organic N fertilizers resulted in short-lasting peaks of CO_2. The average CO_2 values also showed differences among fertilization treatments, with the greatest value in the 150 kg N ha^{-1} organic fertilizer treatment (Table 3). In addition, during the fast-growing period of the crop (February-May), significant differences were also found between N fertilization treatments (Fig. 5B).

Cumulative C losses, grain yield and yield-scaled CH_4 and CO_2 emissions

In the long-term experiment, taking into account the whole period of gas measurements, the soil absorbed 1.07 and 2.40 kg CH_4-C ha^{-1} and emitted 2610.57 and 3984.85 kg CO_2-C ha^{-1} in the CT and NT treatments, respectively, with significant differences between them (Table 2). On the other hand, no significant differences in the absorption/emission of CH_4 and CO_2 were found between N fertilization treatments or the interaction between tillage and N fertilization. Although not significant, we found a trend of lower CH_4 consumption with increasing fertilizer N rates (Table 2). In the short-term experiment, the cumulative absorption of CH_4-C by the soil amounted to 2.69 and 1.16 kg CH_4-C ha^{-1} under CT and NT, respectively, the values being significantly different (Table 3). Significant differences between tillage and N fertilization treatments were also found for cumulative CO_2-C losses. Averaged across fertilizer treatments, CT emitted 3312.67 kg CO_2-C ha^{-1}, while NT emitted 4480.39 kg CO_2-C ha^{-1} (Table 3). Averaged across tillage treatments, the losses of C as CO_2 ranged from 3226.56 kg CO_2-C ha^{-1} for the control treatment to 4585.60 kg CO_2-C ha^{-1} for the 150 kg organic N ha^{-1} treatment (Table 3).

Greater grain production was observed in both experiments under NT. In the long-term one, grain yield in the 2011-12 growing season was 246 and 1554 kg ha^{-1} for the CT and NT treatments, respectively (Table 2). In the short-term experiment, grain yield, expressed as the sum of the 2011-12 and 2012-13 growing seasons, reached 2263 and 5692 kg ha^{-1} for the CT and NT treatments, respectively (Table 3). The application of increasing rates of N significantly increased grain yield in the long-term experiment, with 720, 941 and 1040 kg grain ha^{-1} for the 0, 60 and 120 kg N ha^{-1} treatments, respectively (Table 2). In the short-term experiment, the yields obtained when 75 and 150 kg ha^{-1} of organic N was added as pig slurry (4657 and 5335 kg grain ha^{-1}) were greater than when the same rates were added as mineral N fertilizer (3651 and 3885 kg grain ha^{-1}) (Table 3).

As was explained in the Materials and Methods section, the quotient between the amount of CO_2 equivalent emitted as CH_4 and CO_2 and the production of grain was calculated for each treatment. In the long-term experiment, the NT treatment emitted five times less CO_2 equivalent per kg of grain than the CT treatment (Table 2). In the same experiment, the use of increasing rates of mineral N fertilizer showed no statistical differences between treatments in the CO_2 equivalent emitted per kg of grain, although a trend to a higher efficiency (i.e., less emissions of CO_2 per unit of grain produced) was observed when the amount of N fertilizer applied was increased.

In the short-term experiment, tillage and fertilization both significantly affected the yield-scaled GHG emissions (Table 3). The lowest yield-scaled emissions were found in the NT treatment with either 75 kg mineral N ha^{-1}or 150 kg organic N ha^{-1} (0.47 kg CO_2 equivalent kg $grain^{-1}$), while the highest emissions were found in CT with 75 kg mineral N ha^{-1} (1.64 kg CO_2 equivalent kg $grain^{-1}$) (Table 3). Following the result found in the long-term experiment, in the short-term experiment the NT treatment showed two times less emission of CO_2 equivalent than the CT treatment. Furthermore, the organic fertilizer treatments (75 and 150 kg organic N ha^{-1}) caused lower ratios than the control and the 75 kg mineral N ha^{-1} treatments, while the application of 150 kg mineral N ha^{-1} resulted in intermediate values (Table 3).

Tillage and N fertilization effects on soil C inputs and stocks in the short-term experiment

In the short-term experiment, tillage and N fertilization treatments significantly affected the aboveground C inputs (crop residues), while no differences between treatments were found in the root biomass C inputs (Table 4). As an average of all treatments, the aboveground C inputs accounted for 86.5% of the biomass C inputs to the soil while the root biomass C inputs only accounted for 13.5%. For the three growing seasons studied (2010-2011, 2011-2012 and 2012-2013), the CT and NT treatments resulted in mean aboveground C inputs of 97 and 155 g C m^{-2}, respectively (Table 4). These values imply that the aboveground C inputs are 60% greater under NT than under CT. On average, the application of 150 kg organic N ha^{-1} resulted in the greatest amount of aboveground biomass C inputs (169 g C m^{-2}) and the control treatment in the lowest (93 g C m^{-2}) (Table 4). After three years of contrasting treatments, no differences between tillage and fertilization treatments were observed in SOC stocks (Table 5). Mean SOC stock for the whole soil profile (0-75 cm) expressed on an equivalent soil mass basis was 98.7 and 95.8 Mg C ha^{-1} in the CT and NT treatments, respectively.

Discussion

CH_4 regulating variables

The activity of methanotrophic bacteria is regulated by soil physic-chemical conditions (Bender and Conrad, 1995). However, in our study soil temperature was the only variable that showed a significant relationship with CH_4 fluxes according to the stepwise regression performed, without effects of soil moisture. This result could be explained by the low amount of water present in the soil during most of our experiment, which would not represent a limitation for methanotrophic bacteria.

Tillage effects

In both the long-term and short-term field experiments, the soil acted as a net sink of CH_4. However, we obtained contrasting results between tillage systems, with CH_4 oxidation under NT greater in the long-term experiment and lower in the short-term one. Different authors have suggested that CH_4 oxidation can be reduced by tillage because of its effects on gas diffusivity or because it causes long-term damage to the methanotrophic community (Ball et al. 1999; Hütsch, 2001). These findings suggest that the number of years under NT can influence the methanotrophic capacity of a soil. In an NT chronosequence performed in a dryland area similar to that in the present study, Plaza-Bonilla et al. (2013) found an improvement of soil structure when the number of years under NT increased. Thus, the greater methanotrophic activity found under NT in the long-term experiment might be related to a better soil structure that could counterbalance the higher WFPS found under this system. By contrast, the greater CH_4 oxidation found under CT in the short-term experiment might be explained by its short duration and the possible lack of differences between tillage treatments in soil porous architecture or methanotrophic communities (Hütsch, 1998). Another possible explanation for these contrasting results between the experiments could be the effect of soil texture, which was coarser in the long-term experiment. In a study on the effects of soil texture on CH_4 uptake, Dörr et al. (1993) found that gas permeability was one order of magnitude higher in coarse-textured soils than in fine-textured soils.

The magnitude of CO_2 fluxes in our experiments, with a maximum of 2500 mg CO_2-C m^{-2} d^{-1}, is in line with the values observed by other authors in the Mediterranean area. For instance, under dryland cereal production in central Spain, Meijide et al. (2010) reported a maximum flux of 1102 and 770 mg CO_2-C m^{-2} d^{-1} during the crop growth and fallow periods, respectively. Similarly, values below 2000 mg CO_2-C m^{-2} d^{-1} were reported when CO_2 fluxes were measured under different tillage and cropping systems in a dryland area of NE Spain (Álvaro-Fuentes et al. 2008).

In both experiments, higher CO_2 fluxes and also cumulative CO_2-C losses were observed under NT than under CT. Soil CO_2 emissions are the result of two processes: first, the autotrophic respiration of plant roots, which does not represent a net loss of C from the soil and, second, the heterotrophic respiration of decomposer microorganisms that use SOC as a source of energy for their activity. In the literature, NT has often been claimed as a soil management system that reduces the emission of CO_2 from soils to the atmosphere compared with CT (Kessavalou et al. 1998). However, some authors have found that, as compared with more humid regions, in dryland Mediterranean agroecosystems the use of NT causes greater or equal CO_2 emissions when compared with CT, particularly in dry years (Álvaro-Fuentes et al. 2008b; Morell et al. 2011). The greater CO_2 emissions found under NT could be due to the enhancement of soil respiration and mineralization processes. The higher soil water content under NT could have enhanced microbial activity. In line with this hypothesis, greater microbial biomass C and enzymatic activities under NT than under CT have been found in the Mediterranean area (Madejón et al. 2009; Álvaro-Fuentes et al. 2013).

Though we observed greater CO_2 emissions from the soil to the atmosphere under NT in both experiments, our results showed a five and two times lower yield-scaled CO_2 equivalent under NT than under CT in the long-term and short-term experiments, respectively. These findings demonstrate the need for a holistic evaluation of the GWP of each agricultural management practice, taking into account its associated grain production. Mosier et al. (2006) introduced the concept of greenhouse gas intensity, relating GWP to crop yield. Van Groenigen et al. (2010) pointed out the need to link agronomic productivity and environmental sustainability, postulated that expressing GHG emissions as a function of land area is not helpful and may be counterproductive, and suggested that GHG emissions should be assessed as a function of crop yield. Although the latter authors referred to the effect of nitrogen application on N_2O emissions, our results demonstrate that the concept of yield-scaled emissions can also be applied to other GHG (CH_4 and CO_2) and agricultural management practices such as soil tillage.

Nitrogen type and rate effects

According to our results, the application of pig slurry to the soil led to peaks of CH_4 and CO_2 emissions while the application of mineral fertilizers did not. The instantaneous (i.e., after three hours) increase in the emission of CH_4 after the application of pig slurry implied a change in the role of soil, from CH_4-oxidizer to emitter. This change in the dynamics of CH_4 fluxes could be the result of several processes. First, as an average of all applications, the pig slurry used in our experiment contained about 94% water by weight. Thus, each addition of pig slurry to the soil represented an input of about 3 mm of water. Although this is a relatively small amount, it could have produced anaerobic conditions in some soil microsites, especially in the most superficial soil layer, thus changing them from methanotrophic to methanogenic activity. Also, due to the liquid nature of the organic manure, the NH_4^+ present in the pig slurry could have infiltrated into the soil matrix much faster that in the mineral fertilizer. It is known that the application of NH_4^+ to the soil has an inhibitory effect on the methanotrophic communities as a result of competitive inhibition of methane monooxygenase, the enzyme responsible for CH_4 oxidation (Dunfield and Knowles, 1995; Le Mer and Roger, 2001). The volatilization of the CH_4 dissolved in the slurry and the microbial degradation of short-chained volatile fatty acids present in animal manures have also been pointed out as mechanisms that can produce peaks of CH_4 when pig slurry is applied to the soil (Chadwick et al. 2000).

We found no significant differences in the cumulative losses of C as CO_2 when increasing rates of mineral N were applied in the long-term and short-term experimental fields. By contrast, the application of pig slurry in the short-term experiment led to higher CO_2 fluxes than the application of mineral fertilizer. Moreover, in the short-term experiment, although greater biomass C inputs to the soil were found under organic fertilization than under mineral fertilization, no differences in SOC stocks were found between the two fertilizer types. Plaza et al. (2004) studied the effects of applying increasing rates of pig slurry (from 30 to 150 m^3 ha^{-1} y^{-1}) to the soil in a semiarid area of Spain. They observed no differences in SOC between pig slurry rates and suggested that this result could be attributed to the small amount of organic C and the relatively large N content of that manure, which could lead to microbial oxidation of native soil organic C. Thus, our findings of higher CO_2 emissions and C inputs when using pig slurry and the lack of differences in SOC stocks when compared to the control or the mineral treatments could be explained by an enhanced mineralization of the C contained in the pig slurry. On average, each application of 75 kg N ha^{-1} as pig slurry in the short-term experiment represented an input of 340 kg C ha^{-1}. Thus, during the experimental period 1020 and 2040 kg C ha^{-1} were applied in the 75 and 150 kg organic N ha^{-1} treatments, respectively. Taking into account that these treatments emitted 719 and 784 kg CO_2-C ha^{-1}, respectively, more than their equivalent treatments with mineral N fertilizer, a decomposition of about 30%-70% of the C applied with the pig slurry can be estimated, a range in line with those reported by Rochette and Gregorich (1998) for manured soils. Although pig slurry increased CO_2 emissions when compared with mineral N fertilization, its application reduced the CO_2 equivalent per unit of grain produced, thus showing a lower emission of GHG. However, we found no differences in that ratio between N rates, regardless of the type of N fertilizer applied.

Tillage and nitrogen interaction

The interaction between tillage and N fertilization significantly affected grain yield in both experiments and the amount of aboveground biomass and the yield-scaled emissions only in the short-term one. In Mediterranean areas crop response to N application is usually limited by the availability of water in the soil. Therefore, in these areas, the greater amount of water in the soil when NT is used usually leads to a higher biomass and yield production after N application (Cantero-Martínez et al. 2003). A significant interaction between tillage and N fertilization was also found in the fluxes of CH_4 and CO_2 from the soil to the atmosphere. The higher amount of water in the soil under NT led to greater CH_4 and CO_2 pulses during organic fertilizer application events due to the antagonism between NH_4^+ and low methanotrophic activity and high microbial activity, respectively (Conrad, 1996; Almagro et al. 2009).

Conclusions

The results of this study show that tillage and N fertilization and their interaction affect the soil fluxes of CH_4 and CO_2. The NT treatment led to higher emissions of CO_2 to the atmosphere than the CT treatment. Although in general the soil acted as a CH_4 sink, contrasting tillage effects were found in two experimental fields. Thus, whereas in the long-term experiment greater CH_4 oxidation was observed under NT than under CT, in the short-term experiment, CH_4 oxidation was much lower under NT. The application of pig slurry led to immediate peaks of CH_4 and CO_2 emission fluxes and also enhanced the C lost as CO_2 during the whole experimental period. By contrast, there were no significant differences in the cumulative losses of C as CO_2 when increasing rates of mineral N were applied in both the long-term and the short-term experiments. Compared with CT, the use of NT caused a five- and two-fold reduction in the CO_{2eq} emitted per unit of mass of grain in the long-term and short-term field experiments, respectively. The use of pig slurry also reduced the ratio when compared with the mineral or the control treatments. Our study demonstrates that, in dryland Mediterranean agroecosystems, the combination of NT and medium rates of either mineral or organic N fertilization can be an appropriate management strategy for optimizing CO_2 and CH_4 emissions and grain yield production.

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Figure captions

Fig. 1 Air temperature (continuous line) and rainfall events (bars) in (A) the long-term experiment and (B) the short-term experiment.

Fig. 2 Soil temperature as affected by tillage (CT, conventional tillage; NT, no-tillage) in (A) the long-term experiment and (B) the short-term experiment. Vertical arrows indicate fertilizer applications.

Fig. 3 Soil water-filled pore space as affected by tillage (CT, conventional tillage; NT, no-tillage) in the long-term and the short-term experiments. *Indicates significant differences between tillage treatments for each date at $P<0.05$. Vertical arrows indicate fertilizer applications.

Fig. 4 Soil CH_4 (A) and CO_2 (B) emissions in the short-term experiment as affected by tillage (CT, conventional tillage; NT, no-tillage). *Indicates significant differences between tillage treatments for each date at $P<0.05$. Vertical arrows indicate fertilizer applications.

Fig. 5 Soil CH_4 (A) and CO_2 (B) emissions in the short-term experiment as affected by nitrogen fertilization (0, control; 75 Mineral, 75 kg N ha^{-1} of mineral N; 150 Mineral, 150 kg N ha^{-1} of mineral N; 75 Organic, 75 kg N ha^{-1} as pig slurry; 150 Organic, 150 kg N ha^{-1} as pig slurry). * Indicates significant differences between fertilization treatments for each date at $P<0.05$. Vertical arrows indicate fertilizer applications.

Fig. 6 Regression analysis between soil temperature and CH_4 flux. Each point represents the average of all treatments for each sampling date. Data from samplings performed three hours after each fertilizer application are excluded in order to avoid the effect of N on CH_4 oxidation.

Fig. 7 Soil CO_2 emissions in the long-term experiment as affected by tillage (CT, conventional tillage; NT, no-tillage). * Indicates significant differences between tillage treatments for each date at $P<0.05$. Vertical arrows indicate fertilizer applications.

Table 1. Composition of the pig slurry used in the organic fertilization treatment as pre-seeding and top-dressing applications in the short-term experiment during the three growing seasons studied (2010-2011, 2011-2012, 2012-2013) (values in g kg^{-1} dry weight).

Pig slurry characteristics	2010-2011		2011-2012		2012-2013	
	Pre-seeding	Top-dressing	Pre-seeding	Top-dressing	Pre-seeding	Top-dressing
Dry matter	45.0	94.0	19.0	19.5	56.0	138.0
Organic C*	412.5	nd	337.0	392.5	391.5	396.0
Kjeldahl N*	34.2	23.6	29.8	34.2	24.2	23.6
Ammonium N	44.5	33.0	104.9	125.5	36.4	42.5
P	18.7	19.3	16.9	17.1	16.8	18.7
K	22.6	18.2	77.4	81.45	27.9	26.5

* Values of the dry residue

nd: not determined

Table 2. Analysis of variance of water-filled pore space (WPFS) (%), fluxes of CH_4 and CO_2 (mg CH_4-C m^{-2} d^{-1} and mg CO_2-C m^{-2} d^{-1}, respectively), cumulative C losses for both gases during the whole experimental period (kg C ha^{-1}), 2011-2012 grain yield (kg ha^{-1} at 10% moisture) and ratio between the CH_4 and CO_2 losses expressed in CO_2 equivalent and grain yield in the 2011-2012 growing season as affected by tillage (CT, conventional tillage; NT, no-tillage), N fertilization (0, control; 60, mineral N at 60 kg N ha^{-1}; 120, mineral N at 120 kg N ha^{-1}), date of sampling and their interactions in the long-term field experiment. Values of gas fluxes and WFPS are the means of all samplings.

Effects	Long-term experiment						
	WFPS	Gas fluxes		Cumulative C flux		2011-12 Grain yield	kg CO_2 eq. kg $grain^{-1}$
		CH_4	CO_2	CH_4	CO_2		
CT	19.84 b¶	-0.249 a	516.29 b	-1.065 a	2610.57 b	245.8 b	4.64 a
NT	44.08 a	-0.424 b	779.33 a	-2.396 b	3984.85 a	1554.3 a	0.91 b
0	32.07	-0.443	617.00	-2.249	3139.09	719.6 c	3.51
60	31.55	-0.341	667.89	-1.700	3338.43	940.7 b	2.90
120	32.27	-0.230	663.21	-1.242	3415.62	1039.8 a	1.92
CT – 0	19.32	-0.390	462.21	-1.747	2370.03	178.4 c	6.00
CT – 60	22.21	-0.183	551.47	-0.726	2716.57	226.8 c	4.97
CT – 120	17.99	-0.174	535.63	-0.726	2745.12	332.1 c	2.95
NT – 0	44.82	-0.495	768.85	-2.751	3908.16	1260.8 b	1.01
NT – 60	40.89	-0.494	779.89	-2.676	3960.29	1654.5 a	0.83
NT – 120	46.54	-0.285	789.19	-1.760	4086.11	1747.5 a	0.89
ANOVA							
Tillage	*<0.001*	*0.009*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*
Nitrogen	*0.191*	*0.068*	*0.739*	*0.061*	*0.748*	*<0.001*	*0.072*
Date	*<0.001*	*0.011*	*<0.001*				
Tillage x Nitrogen	*0.550*	*0.435*	*0.875*	*0.427*	*0.922*	*<0.001*	*0.094*
Tillage x Date	*<0.001*	*0.488*	*<0.001*				
Nitrogen x Date	*0.995*	*0.846*	*0.844*				
Tillage x Nitrogen x Date	*0.021*	*0.324*	*0.529*				

¶ For each variable, different letters indicate significant differences between treatments at P<0.05.

Table 3. Analysis of variance of water-filled pore space (WPFS) (%), fluxes of CH_4 and CO_2 (mg CH_4-C m^{-2} d^{-1} and mg CO_2-C m^{-2} d^{-1}, respectively), cumulative C losses for both gases during the whole experimental period (kg C ha^{-1}), 2011-2012 plus 2012-2013 grain yield (kg ha^{-1} at 10% moisture) and the ratio between the loss of CH_4 and CO_2 expressed in CO_2 equivalent and grain yield (sum of the 2011-2012 and 2012-2013 growing seasons) as affected by tillage (CT, conventional tillage; NT, no-tillage), N fertilization (0, control; 75 Min, mineral N at 75 kg N ha^{-1}; 150 Min, mineral N at 150 kg N ha^{-1}; 75 Org, organic N as pig slurry at 75 kg N ha^{-1} and 150 Org, organic N as pig slurry at 150 kg N ha^{-1}), date of sampling and their interactions in the short-term field experiment. Values of gas fluxes and WFPS are the means of all samplings.

Effects	Short-term experiment						
	WFPS	Gas fluxes		Cumulative C flux		2011-13 grain yield	kg CO_2 eq. kg $grain^{-1}$
		CH_4	CO_2	CH_4	CO_2		
CT	18.47 b¶	-0.281 b	455.99 b	-2.690 b	3312.67 b	2262.8 b	1.07 a
NT	32.01 a	-0.062 a	627.08 a	-1.161 a	4480.39 a	5692.3 a	0.51 b
0	24.29	-0.287	425.21 c	-2.436	3226.56 c	2358.6 d	1.00 a
75 Min	24.54	-0.250	461.29 c	-2.073	3574.64 bc	3651.1 c	1.05 a
150 Min	26.11	-0.191	486.52 c	-1.827	3802.07 bc	3885.2 c	0.72 ab
75 Org	25.78	-0.176	607.85 b	-2.055	4293.78 ab	4657.4 b	0.62 b
150 Org	25.47	0.051	727.55 a	-1.238	4585.60 a	5335.4 a	0.55 b
CT – 0	16.35	-0.419	365.68	-3.512	2819.18	992.3 f	1.42 ab
CT – 75 Min	16.16	-0.369	385.82	-2.903	2899.34	1308.3 ef	1.64 a
CT – 150 Min	22.11	-0.173	402.77	-1.914	3130.57	2225.7 de	0.89 bc
CT – 75 Org	18.50	-0.366	480.46	-3.654	3497.46	2755.4 cd	0.75 c
CT- 150 Org	19.22	-0.079	643.77	-1.468	4216.79	4032.6 b	0.63 c
NT – 0	32.23	-0.154	484.51	-1.360	3633.94	3725.0 bc	0.57 c
NT – 75 Min	32.92	-0.136	535.02	-1.243	4249.95	5993.9 a	0.47 c
NT – 150 Min	30.11	-0.208	569.61	-1.739	4473.56	5544.8 a	0.55 c
NT – 75 Org	33.06	0.010	732.80	-0.456	5090.09	6559.5 a	0.49 c
NT- 150 Org	31.72	0.179	810.37	-1.008	4954.40	6638.3 a	0.47 c
				ANOVA			
Tillage	*<0.001*	*0.013*	*<0.001*	*0.006*	*<0.001*	*<0.001*	*<0.001*
Nitrogen	*0.732*	*0.082*	*<0.001*	*0.7*	*<0.001*	*<0.001*	*<0.001*
Date	*<0.001*	*<0.001*	*<0.001*				
Tillage x Nitrogen	*0.057*	*0.567*	*0.384*	*0.378*	*0.426*	*<0.001*	*<0.001*
Tillage x Date	*<0.001*	*0.039*	*<0.001*				
Nitrogen x Date	*<0.001*	*<0.001*	*<0.001*				
Tillage x Nitrogen x Date	*0.034*	*<0.001*	*0.019*				

¶ For each variable, different letters indicate significant differences between treatments at P<0.05.

Table 4. Analysis of variance of aboveground and root biomass C inputs (g C m^{-2}) as affected by tillage (CT, conventional tillage; NT, no-tillage), N fertilization (0, control; 75 Min, mineral N at 75 kg N ha^{-1}; 150 Min, mineral N at 150 kg N ha^{-1}; 75 Org, organic N as pig slurry at 75 kg N ha^{-1} and 150 Org, organic N as pig slurry at 150 kg N ha^{-1}), growing season and their interactions in the short-term field experiment.

	Short-term experiment						
Effects	Aboveground C inputs				Root biomass C inputs		
	2010-11	*2011-12*	*2012-13*	*Mean*	*2011-2012*	*2012-13*	*Mean*
CT	100.79	53.56	179.70	96.90 b	12.87	23.98	17.81
NT	176.01	105.28	233.14	154.93 a	20.46	23.28	21.51
0	81.75 c	59.60	169.31 b	92.56 c	16.02	20.13	17.67
75 Min	184.31 ab	90.23	164.56 b	132.33 b	15.80	12.00	14.28
150 Min	125.45 abc	92.29	224.51 ab	133.64 b	16.53	30.41	21.16
75 Org	106.58 bc	48.93	202.69 ab	101.78 bc	19.81	35.61	26.99
150 Org	193.90 a	106.05	271.02 a	169.25 a	15.16	19.89	27.60
CT – 0	58.67 c	62.91 abc	151.82 b	84.08 d	15.50	11.34	13.84
CT – 75 Min	115.43 bc	20.83 c	147.44 b	76.13 d	7.16	9.57	8.12
CT – 150 Min	83.72 c	91.86 abc	200.26 b	116.93 cd	12.20	32.60	20.36
CT – 75 Org	92.42 c	26.31 c	212.88 b	89.48 cd	16.24	40.20	28.22
CT- 150 Org	153.71 abc	65.87 abc	186.12 b	117.89 cd	13.26	20.04	16.65
NT – 0	104.83 bc	56.28 abc	186.81 b	101.05 cd	16.54	28.92	21.49
NT – 75 Min	253.19 a	159.63 a	181.68 b	188.53 ab	24.45	14.43	20.44
NT – 150 Min	167.18 abc	92.72 abc	248.77 ab	150.35 bc	20.87	26.03	22.16
NT – 75 Org	120.75 abc	71.54 abc	192.51 b	114.09 cd	23.37	28.73	25.51
NT- 150 Org	234.10 ab	146.22 ab	355.92 a	220.62 a	17.05	19.65	18.09
			ANOVA				
Tillage		*<0.001*				*0.191*	
Nitrogen		*<0.001*				*0.086*	
Growing season (GS)		*<0.001*				0.073	
Tillage x Nitrogen		*<0.001*				*0.630*	
Tillage x GS		*0.455*				*0.323*	
Nitrogen x GS		*<0.001*				*0.349*	
Tillage x Nitrogen x GS		*0.033*				*0.447*	

¶ For each variable, different letters indicate significant differences between treatments at *P*<0.05.

Table 5 Soil organic carbon stock expressed on an equivalent mass basis (SOC_{esm}) as affected by tillage (CT, conventional tillage; NT, no-tillage) and N fertilization (0, control; 75 Min, mineral N at 75 kg N ha^{-1}; 150 Min, mineral N at 150 kg N ha^{-1}; 75 Org, organic N as pig slurry at 75 kg N ha^{-1} and 150 Org, organic N as pig slurry at 150 kg N ha^{-1}) in the short-term field experiment.

Soil depth (cm)	SOC_{esm} stock (Mg C ha^{-1})											
	CT						NT					
	0	75 Min	150 Min	75 Org	150 Org	Mean	0	75 Min	150 Min	75 Org	150 Org	Mean
0-10	17.9 (2.4)*	16.9 (2.5)	17.2 (2.5)	21.1 (3.6)	19.3 (4.1)	18.5 (3.1)	21.2 (1.7)	19.7 (6.0)	19.5 (3.3)	21.3 (8.1)	20.9 (6.0)	20.5 (4.7)
10-75	78.4 (10.6)	70.5 (17.2)	83.1 (13.1)	88.7 (5.1)	80.3 (10.7)	80.2 (11.9)	61.2 (17.7)	80.9 (19.0)	71.9 (20.6)	76.5 (7.8)	85.7 (1.1)	75.3 (15.5)
0-75	96.2 (12.9)	87.4 (18.0)	100.2 (14.5)	109.8 (8.7)	99.7 (7.7)	98.7 (13.3)	82.5 (17.0)	100.6 (24.4)	91.4 (23.9)	97.8 (15.9)	106.6 (6.7)	95.8 (18.0)

* Values in parentheses are the standard deviations of the mean.

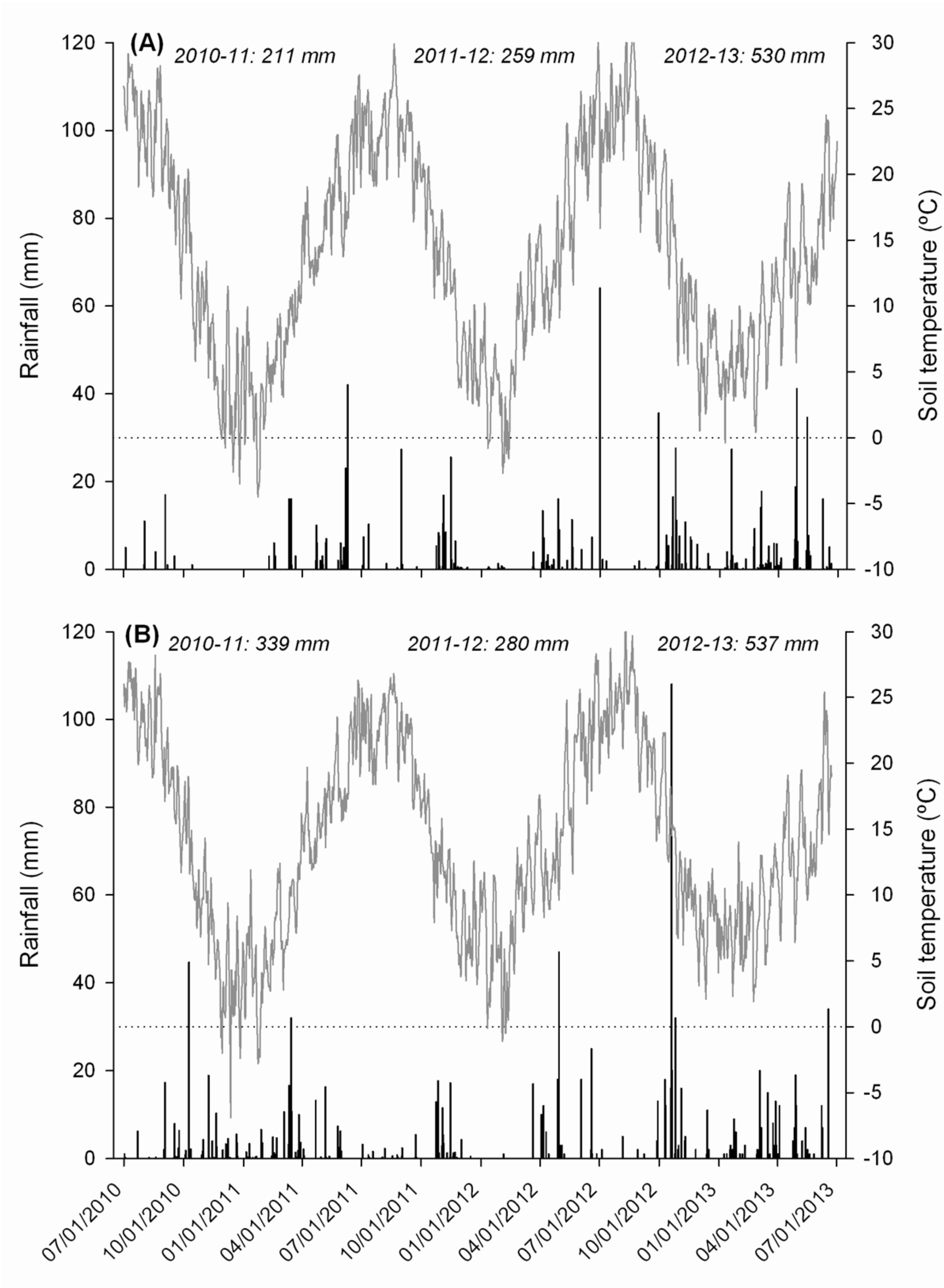

(A)
2010-11: 211 mm
2011-12: 259 mm
2012-13: 530 mm
Rainfall (mm)
Soil temperature (ºC)
0
20
40
60
80
100
120
-10
-5
0
5
10
15
20
25
30
(B)
2010-11: 339 mm
2011-12: 280 mm
2012-13: 537 mm
Rainfall (mm)
Soil temperature (ºC)
0
20
40
60
80
100
120
-10
-5
0
5
10
15
20
25
30
07/01/2010
10/01/2010
01/01/2011
04/01/2011
07/01/2011
10/01/2011
01/01/2012
04/01/2012
07/01/2012
10/01/2012
01/01/2013
04/01/2013
07/01/2013

Fig. 1

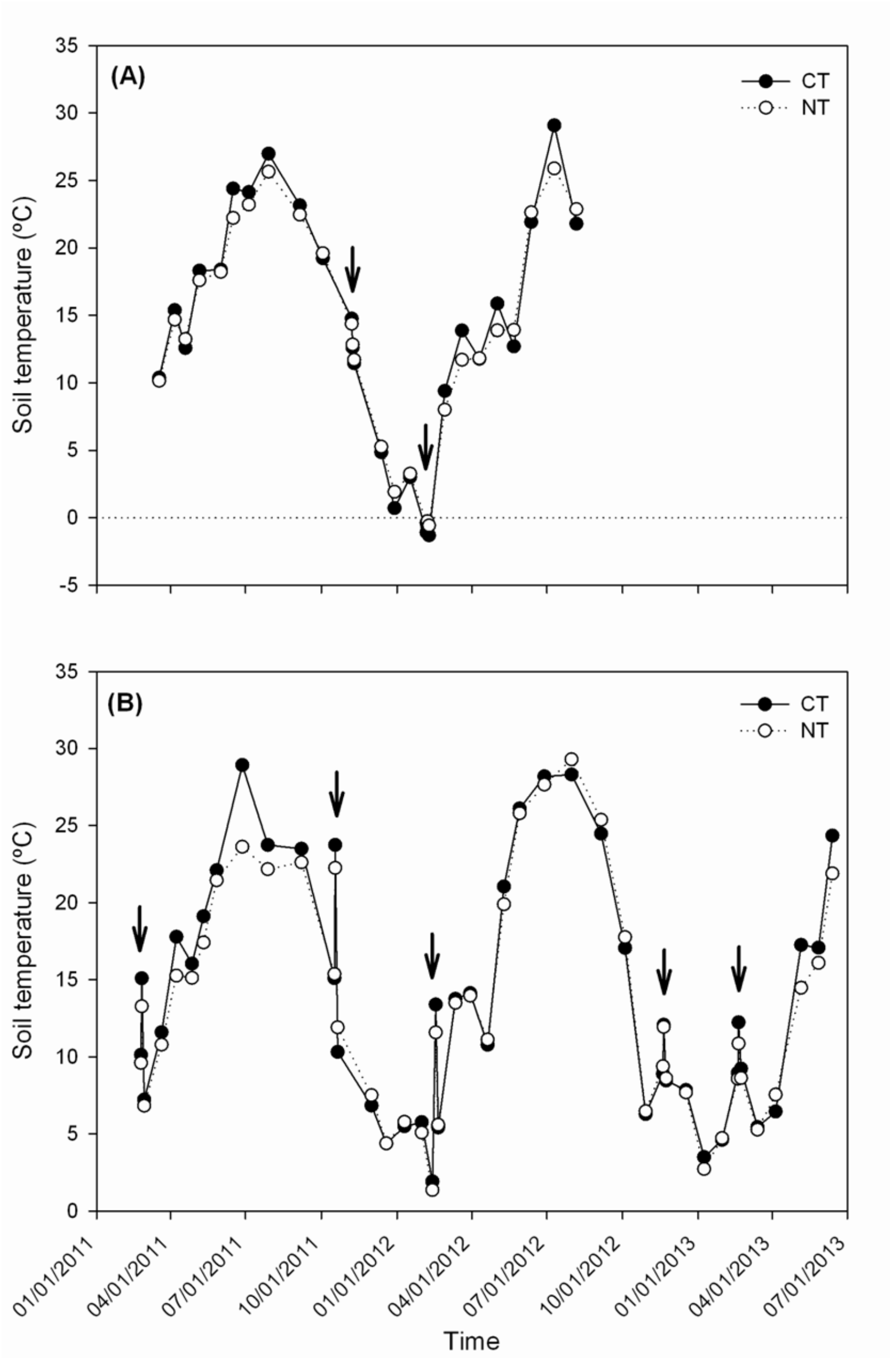

(A)
CT
NT
Soil temperature (ºC)
35
30
25
20
15
10
5
0
-5
(B)
CT
NT
Soil temperature (ºC)
35
30
25
20
15
10
5
0
01/01/2011
04/01/2011
07/01/2011
10/01/2011
01/01/2012
04/01/2012
07/01/2012
10/01/2012
01/01/2013
04/01/2013
07/01/2013
Time

Fig. 2

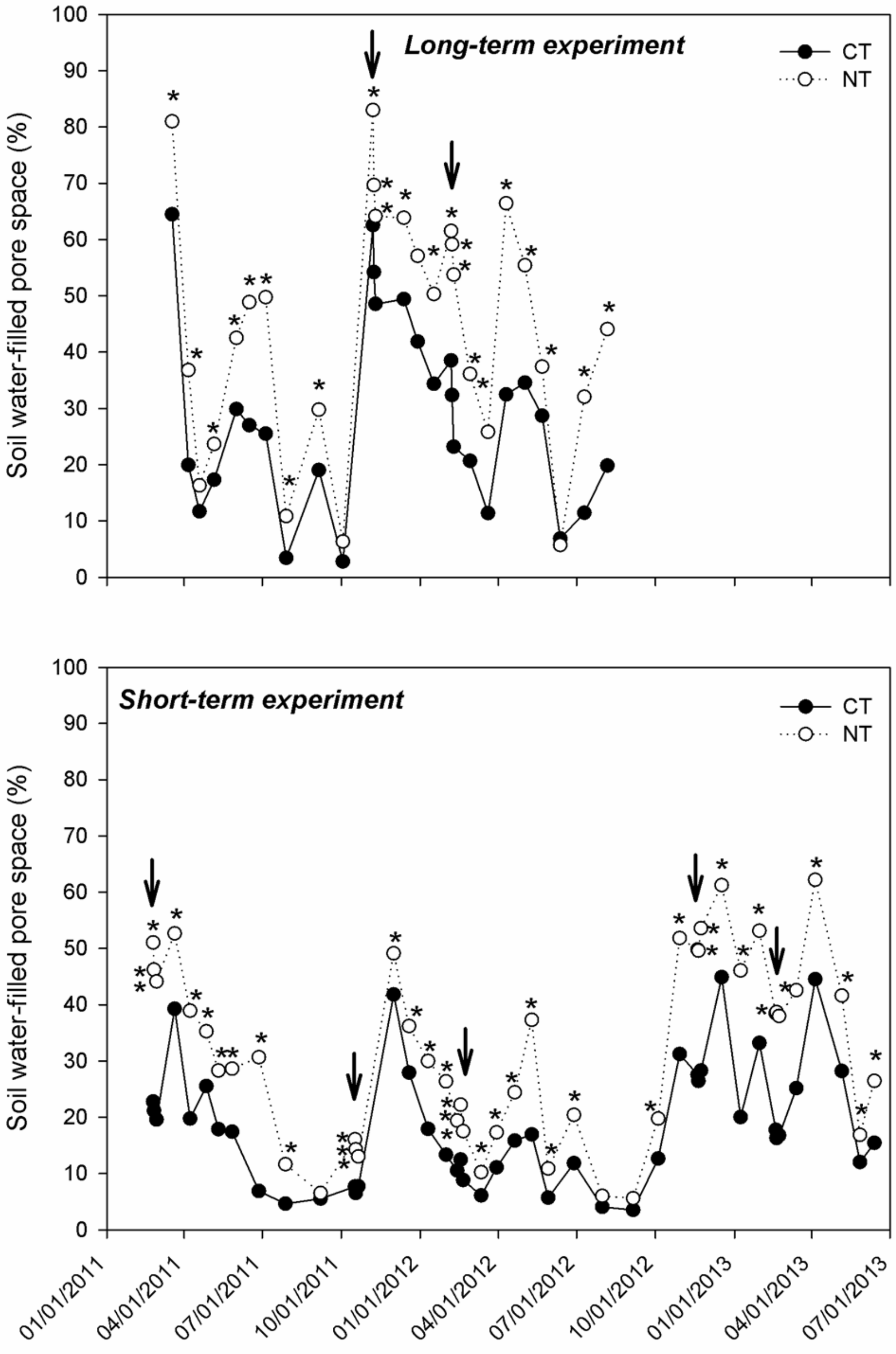

Long-term experiment
CT
NT
Soil water-filled pore space (%)
Short-term experiment
CT
NT
Soil water-filled pore space (%)
01/01/2011
04/01/2011
07/01/2011
10/01/2011
01/01/2012
04/01/2012
07/01/2012
10/01/2012
01/01/2013
04/01/2013
07/01/2013
Time

Fig. 3

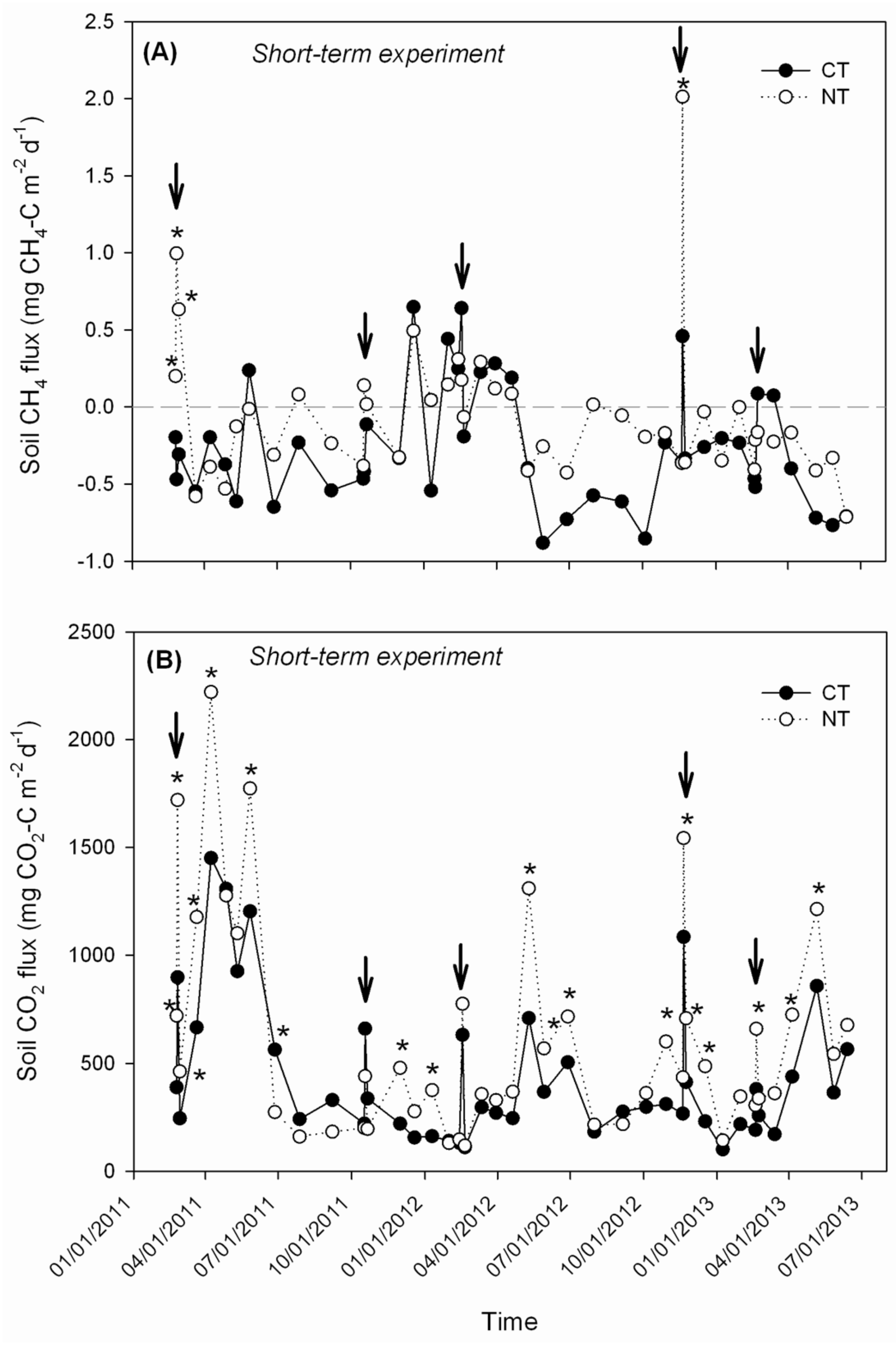

(A)
Short-term experiment
CT
NT
Soil CH_4 flux (mg CH_4-C m^{-2} d^{-1})
(B)
Short-term experiment
CT
NT
Soil CO_2 flux (mg CO_2-C m^{-2} d^{-1})
Time

Fig. 4

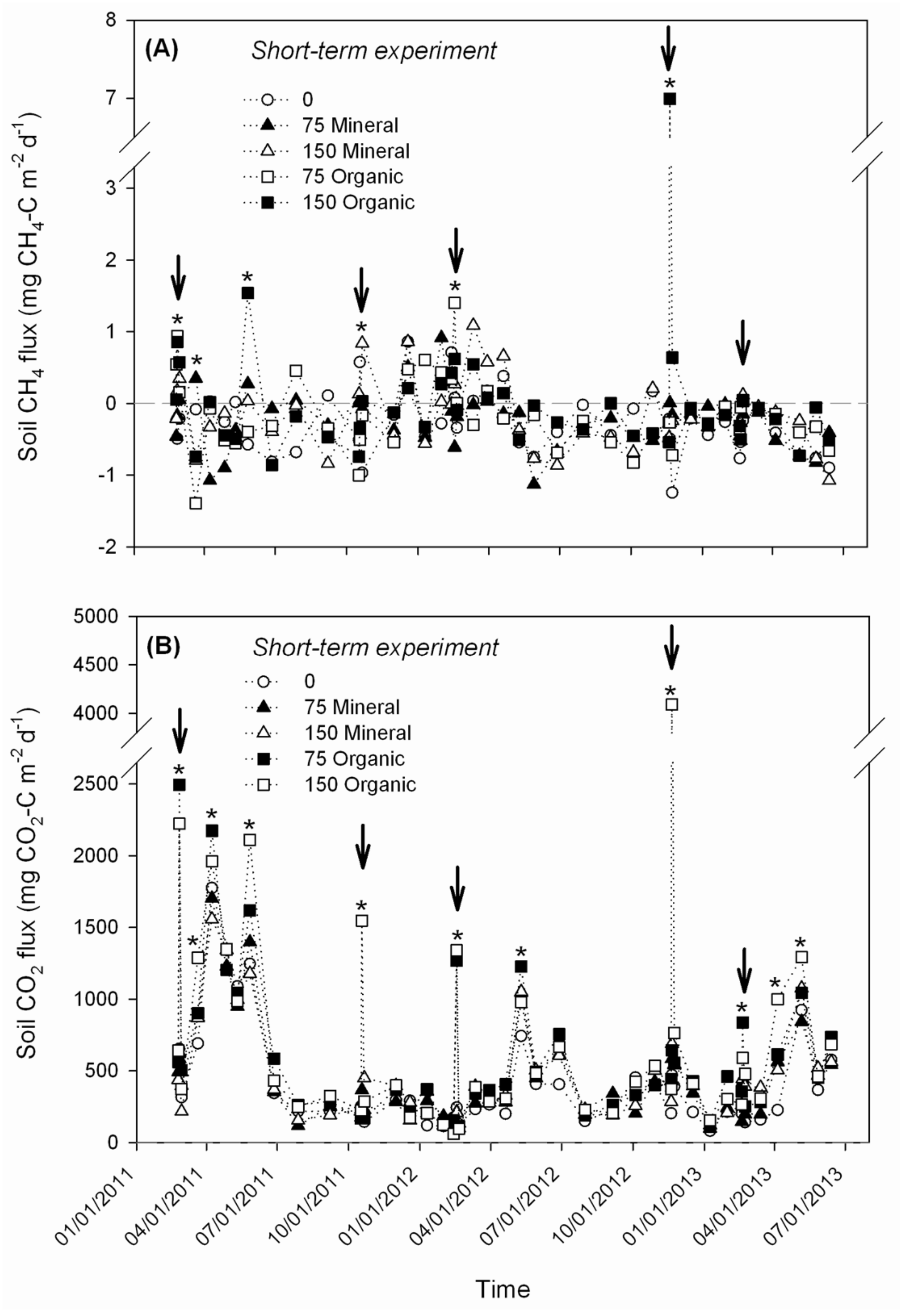

(A)
Short-term experiment
0
75 Mineral
150 Mineral
75 Organic
150 Organic
Soil CH4 flux (mg CH4-C m-2 d-1)
(B)
Short-term experiment
0
75 Mineral
150 Mineral
75 Organic
150 Organic
Soil CO2 flux (mg CO2-C m-2 d-1)
01/01/2011
04/01/2011
07/01/2011
10/01/2011
01/01/2012
04/01/2012
07/01/2012
10/01/2012
01/01/2013
04/01/2013
07/01/2013
Time

Fig. 5

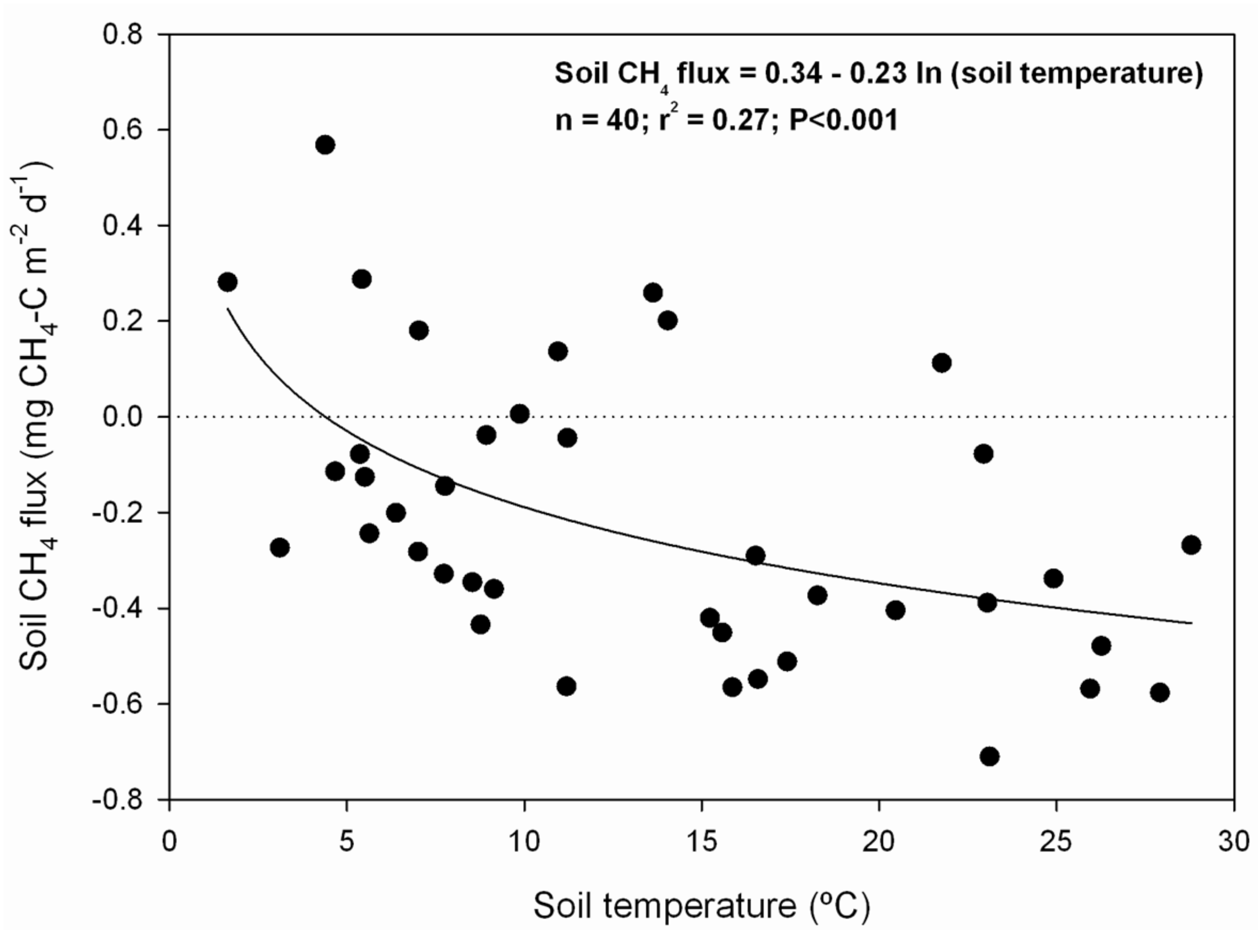
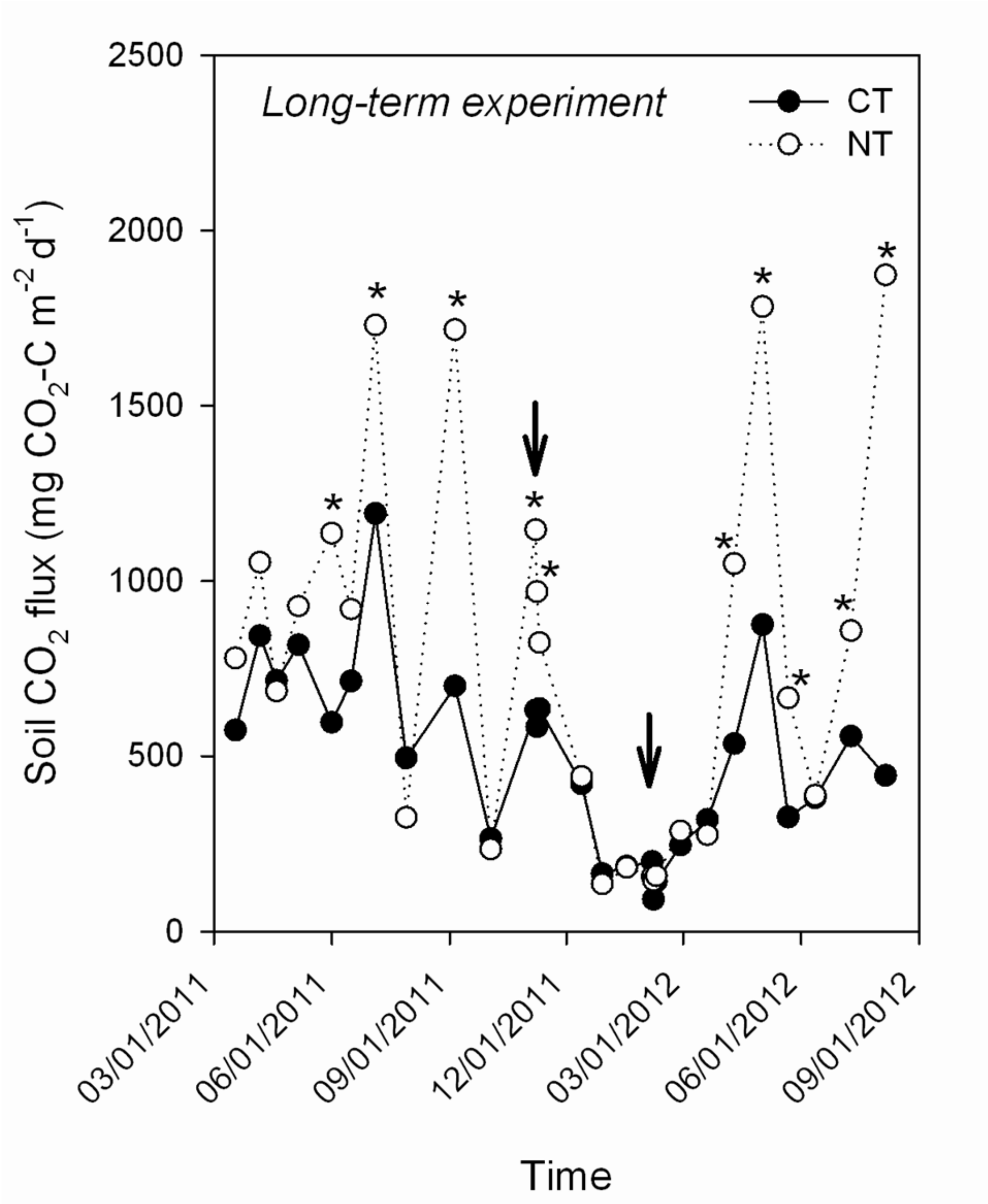

Soil CH_4 flux = 0.34 - 0.23 ln (soil temperature)
n = 40; r^2 = 0.27; P<0.001
Soil CH_4 flux (mg CH_4-C m^{-2} d^{-1})
Soil temperature (ºC)
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
0
5
10
15
20
25
30

Fig. 6



910

911 **Fig. 7**

912